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Essential oils of *Phlomis leucophracta*, *Phlomis chimerae* and *Phlomis grandiflora* var. *grandiflora* from Turkey

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Abstract

The essential oils of three species of *Phlomis* from Turkey, *Phlomis leucophracta*, *Phlomis chimerae* and *Phlomis grandiflora* var. *grandiflora* have been studied. The main constituents of *P. leucophracta* essential oil were β-caryophyllene (20.2%), α-pinene (19.2%) and limonene (11.0%). This species also contained three diterpene derivatives, 15-isopimaradiene, manoyl oxide and *epi*-13-manoyl oxide that summed 1.4%. In *P. chimerae* the principal compounds were β-caryophyllene (31.6%), α-pinene (11.0%), germacrene D (6.1%), limonene (5.5%) and

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linalool (4.7%). In *P. grandiflora* var. *grandiflora*, germacrene D (45.4%), β-caryophyllene (22.8%) and bicyclogermacrene (4.9%) were among the principal derivatives.

Keywords: *Phlomis leucophracta*; *Phlomis chimerae*; *Phlomis grandiflora* var. *grandiflora*; Essential oil; β-Caryophyllene; α-Pinene; Germacrene D; Limonene

1. Introduction

The genus *Phlomis* (Lamiaceae) was revised by Huber-Morath (1982) for the Flora of Turkey (34 species and 10 hybrid).

Phlomis grandiflora H.S. Thompson var. *grandiflora* is a shrub up to 200 cm high, with yellow flowers. This plant prefers *Pinus brutia* forests, *Quercus* scrubs, macchie, limestone slopes and rocks, from 600 to 1200 m. above the sea level.

Phlomis leucophracta P.H. Davis & Hub.-Mor. is a shrub up to 150 cm high, with a brownish upper lip of corolla and a yellow lower lip. It prefers limestone rocks, metamorphic slopes, macchie, *Quercus* scrub and follow fields from sea level up to 1100 m of altitude.

Phlomis chimerae Boissieu is a dwarf shrub up to 30 cm high, and a yellow corolla. This plant lives preferentially in *P. brutia* forest, macchie and rocky slopes from sea level up to 150 m altitude.

All three species are endemic to Anatolia.

Previous studies on the volatiles from *Phlomis* included *Phlomis olivieri*, *Phlomis fruticosa*, *Phlomis lanata* and *Phlomis younghunsbandii*. In *P. olivieri*, Ghassemi et al. (2001) found hexahydrofarnesylacetone, spathulenol, germacrene D, β-caryophyllene and caryophyllene oxide as main constituents. This oil was characterized by a high content of sesquiterpenes and trace amounts of monoterpenes. In another report on the essential oil of the aerial parts of *P. olivieri*, the main compounds were germacrene D, β-caryophyllene, α-pinene and β-selinene (Mirza and Nik, 2003).

In the essential oil obtained from the leaves of *P. fruticosa* collected in Montenegro, β-caryophyllene, (*E*)-methyl-isoeugenol and α-asarone were found as main components (Sokovic et al., 2002a). The antimutagenic activity of the essential oil and crude extract of this species was evaluated by the same research group (Sokovic et al., 2002b). The flowers of *P. fruticosa* collected in Greece yielded an essential oil rich in germacrene D, γ-bisabolene, α-pinene and β-caryophyllene (Tsitsimi et al., 2000).

The main chemicals identified in the essential oil of the aerial parts of *P. lanata* were α-pinene, limonene and β-caryophyllene (Couladis et al., 2000). The authors also tested the oil for its activity against bacteria and fungi. The antimicrobial activity of the essential oil of this species, as well as the effectiveness of the EtOH extract, was also evaluated by Ristic et al. (2000).

Finally, the essential oil of *P. younghunsbandii* contained non-terpenic derivatives such as eugenol, hexadecanoic acid, 9,12-octadecadienoic acid methyl ester and guaiol as principal constituents (Wang et al., 2002). Among non-volatile derivatives, neolignan glucosides were characterized from *P. chimerae* (Ersoz et al., 2002). Furthermore, the anti-ulcerogenic activity of *P. grandiflora* is reported by the Turkish folk medicine (Gurbuz et al., 2003). This is the first report about the composition of the essential oil of these three species of *Phlomis*.

2. Materials and methods

The flowered aerial parts of *P. grandiflora* var. *grandiflora* were collected in Turkey, C2 Antalya, Elmalı district, between Elmalı and Finike, on rocky slopes, 1050 m above the sea level, at the middle of May 2003 (36° 35′ 609″N, 29° 57′ 533″E). The flowered aerial parts of *P. leucophracta* were collected in Turkey, C4 Antalya, Alanya district, Alanya Castle, roadside, 40 m above the sea level, at the middle of May 2003 (36° 32′ 525″N, 31° 59′ 451″E). The flowered aerial parts of *P. chimerae* were collected in Turkey, C3 Antalya, Tekirova, Çıralı, between Tekirova and Kumluca, in a *P. brutia* forest, 7 m above the sea level at the middle of May 2003 (36° 25′ 446″N, 30° 28′ 366″E). Voucher specimens of *P. grandiflora* var. *grandiflora*, *P. leucophracta* and *P. chimerae* are deposited in the Herbarium of the Biology Department of Akdeniz University at Gokturk 5101, Gokturk 5102 and Gokturk 5104.

The plant material was dried in the shade at room temperature till constant weight and about 100 g was separately hydrodistilled in a Clevenger-type apparatus for 2 h.

The GC analyses were accomplished with an HP-5890 Series II instrument equipped with HP-WAX and HP-5 capillary columns (30 m × 0.25 mm, 0.25 μm film thickness), working with the following temperature program: 60 °C for 10 min, ramp of 5 °C/min up to 220 °C; injector and detector temperatures 250 °C; carrier gas nitrogen (2 ml/min); detector dual FID; split ratio 1:30; injection of 0.5 μl). The identification of the components was performed, for both the columns, by comparison of their retention times with those of pure authentic samples and by mean of their linear retention indices (l.r.i.) relative to the series of *n*-hydrocarbons.

The relative proportions of the essential oil constituents were percentages obtained by FID peak-area normalization.

GC/EIMS analyses were performed with a Varian CP-3800 gas-chromatograph equipped with a DB-5 capillary column (30 m × 0.25 mm; coating thickness 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperatures 220 and 240 °C, respectively; oven temperature programmed from 60 °C to 240 °C at 3 °C/min; carrier gas helium at 1 ml/min; injection of 0.2 μl (10% hexane solution); split ratio 1:30. Identification of the constituents was based on comparison of the retention times with those of authentic samples, comparing their linear retention indices relative to the series of *n*-hydrocarbons, and on computer matching against commercial (NIST 98 and ADAMS) and home-made library mass spectra built up from pure substances and

Table 1
Composition[a] of the essential oils of the aerial parts of *Phlomis leucophracta*, *Phlomis chimerae* and *Phlomis grandiflora* var. *grandiflora*

Constituents	l.r.i.[b]	*Phlomis leucophracta*	*Phlomis chimerae*	*Phlomis grandiflora*
Heptanal	901	0.5	–	–
α-Thujene	933	1.6	0.8	0.2
α-Pinene	941	19.2	11.0	2.4
Sabinene	978	0.5	0.2	–
β-Pinene	981	1.4	0.7	0.2
Myrcene	992	0.5	–	–
2-Pentyl furan	994	0.6	0.4	0.5
α-Phellandrene	1006	0.8	0.3	0.2
α-Terpinene	1020	0.9	0.4	0.2
p-Cymene	1027	0.4	0.3	–
Limonene	1032	11.0	5.5	2.7
1,8-Cineole	1034	0.2	–	–
(*Z*)-β-Ocimene	1041	–	0.4	0.6
(*E*)-β-Ocimene	1052	0.3	0.3	tr[c]
γ-Terpinene	1064	0.4	0.5	–
Terpinolene	1089	1.7	0.8	0.3
2-Nonanone	1093	tr	–	–
n-Undecane	1100	0.7	–	–
Linalool	1101	–	4.7	0.6
Nonanal	1104	8.8	0.9	–
(*E*,*Z*)-2,6-Nonadienal	1158	–	–	tr
(*E*)-2-Nonenal	1165	–	0.2	–
4-Terpineol	1179	0.2	0.7	–
α-Terpineol	1190	0.2	0.9	–
Decanal	1205	1.0	0.1	–
Geraniol	1255	–	0.4	–
(*E*)-2-Decenal	1263	0.5	–	–
2-Undecanone	1292	0.3	–	–
Undecanal	1307	0.5	0.2	–
α-Cubebene	1351	0.3	–	0.4
Cyclosativene	1370	–	0.3	–
α-Copaene	1377	0.3	3.3	1.3
β-Bourbonene	1384	0.2	1.0	0.9
β-Cubebene	1391	0.3	0.3	2.1
Isocaryophyllene	1404	–	0.2	–
α-Gurjunene	1408	–	0.6	0.3
Dodecanal	1410	0.7	–	–
β-Caryophyllene	1420	20.2	31.6	22.8
γ-Elemene	1431	–	–	0.3
α-Guaiene	1440	tr	–	–
(*E*)-β-Farnesene	1457	1.1	0.5	1.0
α-Humulene	1459	2.8	2.2	2.8
Alloaromadendrene	1462	–	1.1	–
γ-Muurolene	1477	tr	–	tr
Germacrene D	1482	4.5	6.1	45.4
β-Selinene	1487	–	1.0	–
cis-β-Guaiene	1492	0.4	–	–
bicyclogermacrene	1494	0.8	–	4.9

Table 1 (*continued*)

Constituents	l.r.i.[b]	*Phlomis leucophracta*	*Phlomis chimerae*	*Phlomis grandiflora*
Viridiflorene	1496	–	1.0	–
α-Muurolene	1499	–	0.2	0.3
β-Bisabolene	1509	–	0.2	2.5
γ-cadinene	1514	tr	tr	–
δ-Cadinene	1524	0.4	5.0	1.3
trans-Nerolidol	1563	–	0.1	–
Spathulenol	1578	0.3	–	0.4
Caryophyllene oxide	1583	1.7	4.8	0.4
T-Cadinol	1642	–	0.2	–
T-Muurolol	1643	–	0.8	–
α-Eudesmol	1653	–	tr	–
α-Cadinol	1656	–	0.4	–
β-Bisabolol	1673	tr	1.6	–
Benzyl benzoate	1766	–	tr	tr
Hexahydrofarnesylacetone	1843	–	0.4	–
n-Nonadecane	1900	tr	–	–
15-Isopimaradiene	1964	0.4	–	–
Manoyl oxide	1992	0.5	–	–
epi-13-Manoyl oxide	2013	0.5	–	–
Total identified		87.6	92.6	95.0
Yield (% w/w)		0.08	0.11	0.05

[a] Percentages obtained by FID peak-area normalization.
[b] Linear retention indices (HP-5 column).
[c] tr $< 0.1\%$.

components of known oils and MS literature data (Adams, 1995; Davies, 1990; Jennings and Shibamoto, 1980; Massada, 1976; Stenhagen et al., 1974; Swigar and Silverstein, 1981). Moreover, the molecular weights of all the identified substances were confirmed by GC/CIMS, using MeOH as CI ionizing gas.

3. Results and discussion

Sixty-seven compounds, which accounted for 87.3–95.0% of the total compositions of each oil are reported in Table 1.

In *P. leucophracta* monoterpenes represented 38.9% of the whole essential oil: the main ones were α-pinene (19.2%) and limonene (11.0%). In this species the percentage of sesquiterpenes was quite comparable (32.7%), with β-caryophyllene and germacrene D as principal components (20.2% and 4.5%, respectively). Distinctive of this species only was the presence of three diterpene derivatives, 15-isopimaradiene, manoyl oxide and *epi*-13-manoyl oxide that summed 1.4%. A considerable percentage of the oil (13.6%) is due to other non-terpenic compounds, such as straight-chain hydrocarbons, aldehydes, ketones, etc. Among them nonanal was the main one (8.8%).

In *P. chimerae*, α-pinene (11.0%), limonene (5.5%) and linalool (4.7%) were the principal monoterpenes, which accounted for 27.9% of the whole oil. Sesquiterpene percentage (62.5%) was about two-times that of monoterpenes, with β-caryophyllene (31.6%), germacrene D (6.1%), δ-cadinene (5.0%) and caryophyllene oxide (4.8%) as main compounds. No diterpenes at all have been identified in the essential oil, while other non-terpenic compounds, this time, represented only 2.3% of the composition.

In *P. grandiflora* var. *grandiflora*, monoterpenes were little represented (7.4%), with α-pinene (2.4%) and limonene (2.7%) as main constituents. On the contrary, sesquiterpenes constituted the main derivatives of the essential oil (87.1%), with germacrene D (45.4%), β-caryophyllene (22.8%) and bicyclogermacrene (4.9%) among the principal ones. Also in this species diterpene were absent and other non-terpenic compounds were detected only in very small amounts (0.5%).

In the essential oil of these three species of *Phlomis*, the main monoterpenes were α-pinene and limonene and the main sesquiterpenes were β-caryophyllene and germacrene D. Observing the main compounds (α-pinene and limonene among monoterpenes and β-caryophyllene and germacrene D among sesquiterpenes), these three species seem chemically similar. However, many differences can be noted in the percentage distribution of mono- and sesquiterpenes and in the presence of characteristic terpenes in *P. leucophracta* together with non-terpene derivatives. Furthermore, in the oils from *P. chimerae* and *P. grandiflora*, diterpenes were not detected.

With respect to the previously studied species, many differences can be noted, mainly for the presence of phenylpropanoid derivatives and fatty acids methyl esters. All these differences suggest further investigations on other species of *Phlomis* that could represent a biodiversity wealth worth to be studied.

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